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PATENT ABSTRACTS OF JAPAN

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(22) Date of filing: **25.06.1992**

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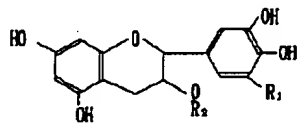
(54) HYALURONIDASE ACTIVITY INHIBITOR

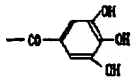
(57) Abstract:

PURPOSE: To obtain a hyaluronidase activity inhibitor containing tea polyphenols such as epigallocatechin gallate as active ingredients, capable of inhibiting hyaluronidase activity having inflammatory action and useful as an anti- inflammatory agent, antiallergic agent, etc.

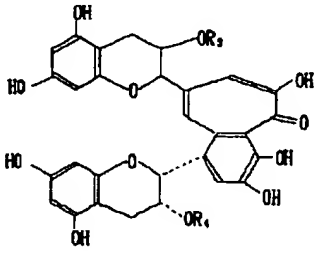
CONSTITUTION: The hyaluronidase activity inhibitor useful for medicine, etc., for reducing various kinds of inflammations and allergies is obtained by using one or more kinds of tea catechins of formula I (R_1 is H or OH; R_2 is H or group of formula II) such as epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin or (+)catechin and tea theaflavin of formula II (R_3 and R_4 are H, group of formula III) such as an isolated type theaflavin, theaflavin monogallate A, theaflavin monogallate B or theaflavin digallate as tea phenols extracted from tea as active ingredients and preparing these tea polyphenols alone or in conbination with vehicles such as gelatin, a solvent such as water or alcohol, diluent, etc., such as carboxymethyl cellulose.



I



II



III

TF - α inflammation

(19) **Publication country** Japan Patent Office (JP)
(12) **Kind of official gazette** Open patent official report (A)
(11) **Publication No.** JP,6-9391,A
(43) **Date of Publication** January 18, Heisei 6 (1994)
(54) **Title of the Invention** Hyaluronidase activity inhibitor
(51) **The 5th edition of International Patent Classification**

A61K 31/35 AED 9360-4C
ABE 9360-4C
ABF 9360-4C
7/00 D 9164-4C
W 9164-4C
7/48 9051-4C
C07D311/62 7252-4C

Request for Examination Un-asking.
The number of claims 2
Number of Pages 4
(21) **Application number** Japanese Patent Application No. 4-190203
(22) **Filing date** June 25, Heisei 4 (1992)
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Address 2-7-23, Seko, Fujieda-shi, Shizuoka-ken
(74) **Attorney**
Patent Attorney
Name Kubota Fujiro

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(57) **Abstract**
Elements of the Invention The hyaluronidase activity inhibitor which contains tea polyphenol as an active principle.
Effect The hyaluronidase activity inhibitor of this invention does not have the worries about a side effect of as opposed to the body as drugs in order to use as a principal component th natural product extracted from the tea by which considerable-amount drink is carried out every day. And the hyaluronidase activity inhibitor of this invention checks activation of hyaluronidase remarkably by low-concentration addition. Therefore, the hyaluronidase activity inhibitor of this invention is used in order to mitigate various inflammation and

allergy as physic, and also it can add for cosmetics etc. and it can prevent disassembly of hyaluronic acid.

Claim(s)

Claim 1 The hyaluronidase activity inhibitor which contains tea polyphenol as an active principle.

Claim 2 The hyaluronidase activity inhibitor according to claim 1 whose tea polyphenol is a least one sort of matter chosen from epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, (+) catechin, isolation mold theaflavin, and theaflavin mono-gallate A, theaflavin mono-gallate B, and theaflavin digallate.

Detailed Description of the Invention

0001

Industrial Application About a hyaluronidase activity inhibitor, this invention acts specifically **it is detailed and** to hyaluronidase, and relates to the hyaluronidase activity inhibitor which contains the tea polyphenol which checks the activation as an active principle.

0002

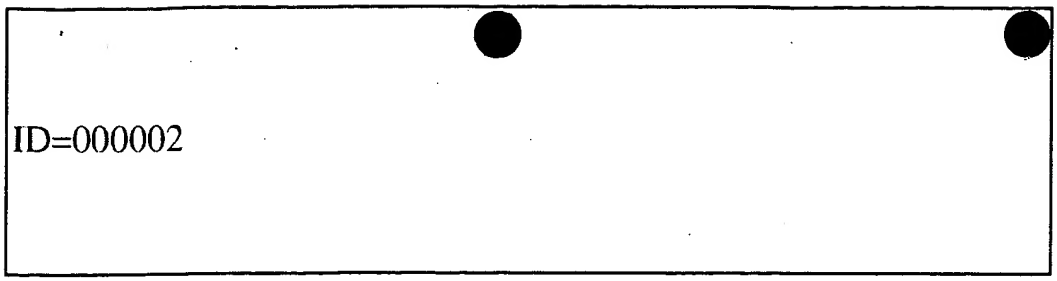
Description of the Prior Art Hyaluronidase is an enzyme which exists in the testis of an animal, snake venom, bacteria, etc., and is the hydrolase of the hyaluronic acid widely distributed over the connective tissue of an animal. It is known that hyaluronidase has the operation as a pathogenic drug. On the other hand, since activity is checked with an anti-inflammatory agent or an antiallergic agent, it is thought possible by checking hyaluronidas activity to mitigate inflammation and allergy. Furthermore, preventing disassembly of the hyaluronic acid mixed by cosmetics, such as a cream, a milky lotion, a lip stick, and a hair product, is also expected. Then, this invention persons tried development of the enzyme inhibitor which checks activation of hyaluronidase and does not have a harmful side effect t the body.

0003

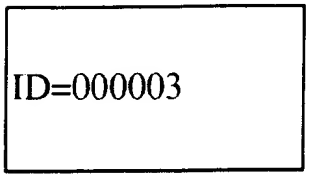
Means for Solving the Problem this invention persons reached **that this matter is contained in tea and tea polyphenol, and** a header and this invention, as a result of repeating research wholeheartedly that not a chemical composition but the matter which ha the drug effect made into the purpose out of a natural product should be searched. That is, this invention offers the hyaluronidase activity inhibitor which contains tea polyphenol as ar active principle. The tea polyphenol which is the principal component of the hyaluronidase activity inhibitor of this invention is tea theaflavin expressed with the tea catechins expressed with the following general formula I, and a general formula II.

0004

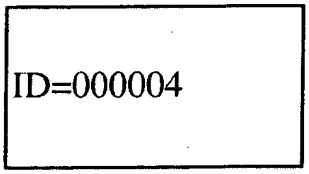
Formula 1



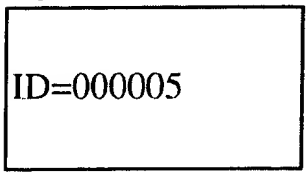
0005 (R1 shows H or OH among a formula, and R2 is H or **0006**.)
Formula 2



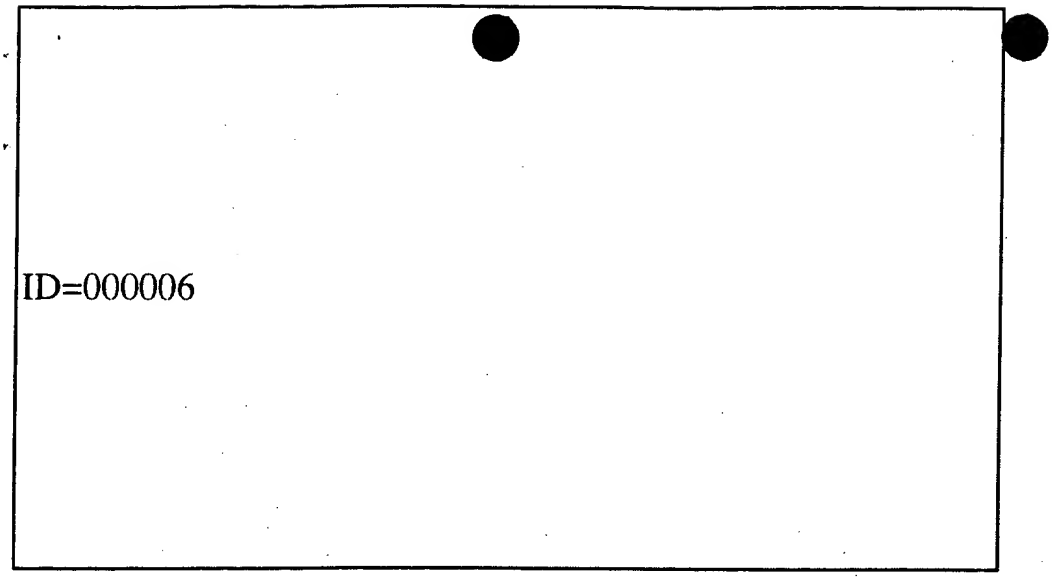
0007) ******.
0008 The following can be mentioned as an example of tea catechins expressed with the above-mentioned general formula I.
(-) Epicatechin (the inside of a general formula I, thing of R1 =H and R2 =H)
(-) Epigallocatechin (the inside of a general formula I, thing of R1 =OH and R2 =H)
(-) Epicatechin gallate (inside **of a general formula I** , and R1 =H, R2 = **0009**)
Formula 3



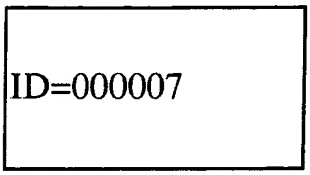
0010 **
(-) Epigallocatechin gallate (the inside of a general formula I, R1 =OH, R2 = **0011**)
Formula 4



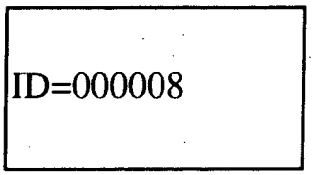
0012 **
General formula II **0013**
Formula 5



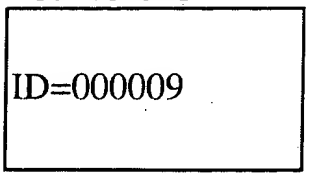
0014 (The inside of a formula, R3, and R4 are H or **0015**.)
Formula 6



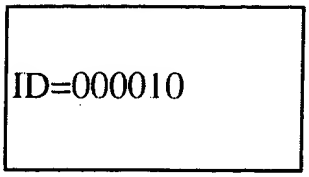
0016 An example and R3 And R4 Even if the same, you may differ.
0017 Next, when the tea theaflavin expressed with the above-mentioned general formula I is shown concretely, there are the following.
Isolation mold theaflavin (the inside of a general formula II, thing of R3 =H and R4 =H)
Theaflavin mono-gallate A (inside **of a general formula II** , and R3 = **0018**)
Formula 7



0019 The thing of R4 =H
Theaflavin mono-gallate B (inside **of a general formula II** , and R3 =H, R4 = **0020**)
Formula 8



0021 **
Theaflavin digallate (the inside of a general formula II, R3, R4 = **0022**)
Formula 9



0023 **
The above-mentioned tea polyphenol can manufacture tea leaves as a raw material, and th process is indicated by JP,59-219384,A, the 60-13780 official report, the 61-130285 officia report, etc.

0024 It is used independently, and also it mixes with a suitable excipient, for example, gelatin, sodium alginate, etc., or the hyaluronidase activity inhibitor of this invention is use combining diluents, such as solvents, such as water and alcohols, and a carboxymethyl cellulose, etc. About the amount of the hyaluronidase activity inhibitor used of this invention, when medicating the body as drugs, the daily dose should just usually take 0.5-10g in taking orally so that it may become about 1-3g preferably. Dosage forms are arbitrary and are used as powder, a tablet, a capsule, etc. Moreover, when blending with cosmetics etc., the last concentration is 1-100 ppm. What is necessary is just to add so tha it may become.

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Example Next, an example explains this invention in detail. In addition, measurement of inhibition ability was performed by the following approach based on the conventional method. Hyaluronidase activity inhibitor solution (1 mM) 50microl Enzyme (from Bovine testis and SIGMA shrine make) solution 100 mul (2,000 unit/ml buffer solution) is added and it is left for 20 minutes at 37 degrees C. next, enzyme activator (trade name: compound 48/80 and SIGMA shrine make) solution (0.1mg **/ml** buffer solution) 100microl i adds -- after leaving it for 20 minutes at 37 degree C, Hyaluronic acid (from rooster comb and WAKO shrine make) solution (0.8 mg/ml buffer solution) 250microl which is a substrate It put in and was left for 40 minutes at 37 degrees C. 0.4N NaOH 100microl Morgan-Elson after stopping a reaction in addition -- strange method (Davidson, E.A., Aronson, N.N.:J.Bio Chem.242, 437 (1967)) of law The quantum of a product is performed. It asked for hyaluronidase activity inhibition ability. Inhibition activity was expressed with the rate of inhibition called for from the following formula. moreover the buffer solution -- 0.1 M The acetic-acid buffer solution (pH 4.0) was used. **0026**

Equation 1 Rate (%) of inhibition =**(A-B)-(C-D)**/(A-B) x100**0027** A: The absorbance B in 585nm of a reference solution : reference solution blank The absorbance D in 585nm of the absorbance C:inhibitor solution in 585nm: Inhibitor solution blank Absorbance in 585nm

0028 The various tea polyphenol shown in the 1st table as an example 1 hyaluronidase activity inhibitor was used, and it asked for the hyaluronidase activity inhibition ability of each matter by the above-mentioned approach. A result is shown in the 1st table.

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Table 1

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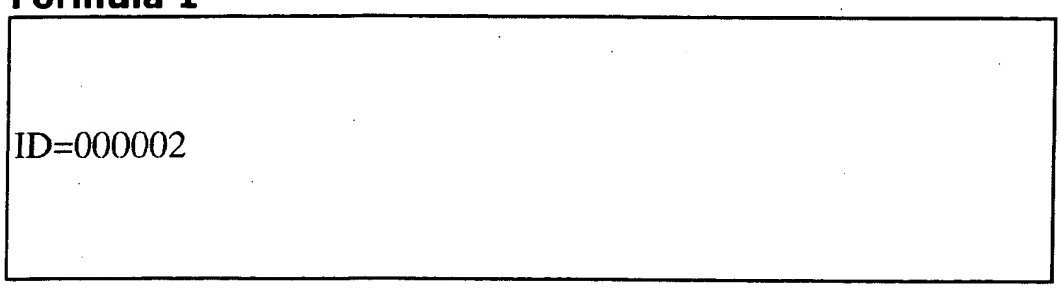

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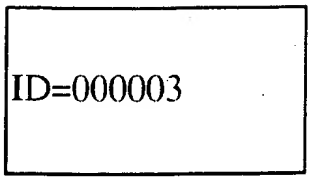

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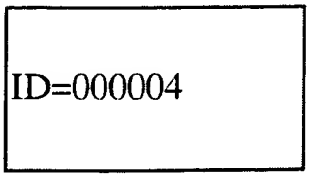

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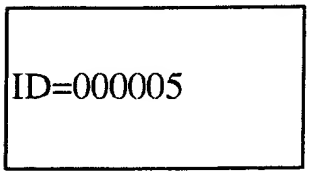

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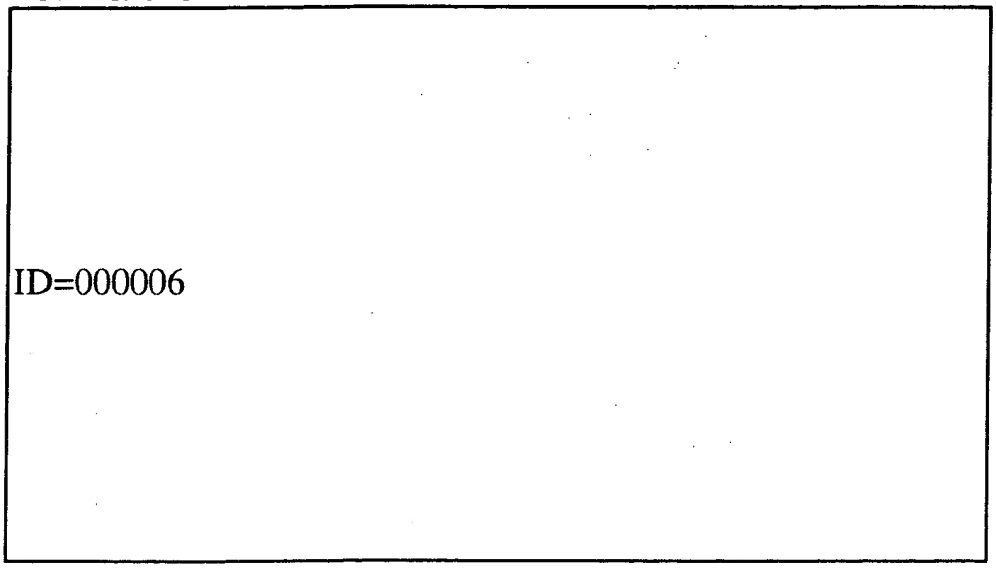

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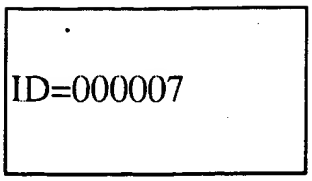

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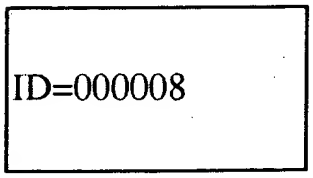

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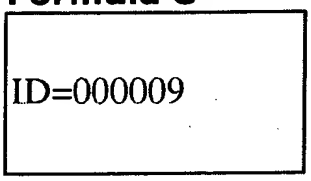

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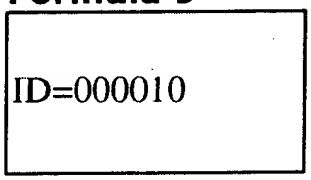

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(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号

特開平6－9391

(43)公開日　平成６年(1994)１月18日

(51)Int.Cl.[5]		識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ａ６１Ｋ	31/35	ＡＥＤ	9360－4C		
		ＡＢＥ	9360－4C		
		ＡＢＦ	9360－4C		
	7/00	Ｄ	9164－4C		
		Ｗ	9164－4C		

審査請求　未請求　請求項の数２(全　４　頁)　最終頁に続く

(21)出願番号　特願平4－190203

(22)出願日　平成４年(1992)６月25日

(71)出願人　591039137
三井農林株式会社
東京都中央区日本橋室町３丁目１番20号
(72)発明者　原　征彦
静岡県藤枝市南駿河台２－２－７
(72)発明者　本田　美和
静岡県藤枝市瀬古２－７－23
(74)代理人　弁理士　久保田　藤郎

(54)【発明の名称】　ヒアルロニダーゼ活性阻害剤

(57)【要約】
【構成】　茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤。
【効果】　本発明のヒアルロニダーゼ活性阻害剤は、日常相当量飲用されている茶から抽出される天然物を主成分とするため、薬剤としても人体に対する副作用の心配がない。しかも、本発明のヒアルロニダーゼ活性阻害剤は低濃度の添加でヒアルロニダーゼの活性化を著しく阻害する。したがって、本発明のヒアルロニダーゼ活性阻害剤は医薬として各種炎症やアレルギーを軽減するために用いる他、化粧品等に添加してヒアルロン酸の分解を防ぐことができる。

【特許請求の範囲】

【請求項１】　茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤。

【請求項２】　茶ポリフェノール類が、エピガロカテキンガレート，エピカテキンガレート，エピガロカテキン，エピカテキン，（＋）カテキン，遊離型テアフラビン，テアフラビンモノガレートＡ，テアフラビンモノガレートＢ及びテアフラビンジガレートの中から選ばれた少なくとも１種の物質である請求項１記載のヒアルロニダーゼ活性阻害剤。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、ヒアルロニダーゼ活性阻害剤に関し、詳しくはヒアルロニダーゼに特異的に作用して、その活性化を阻害する茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤に関する。

【０００２】

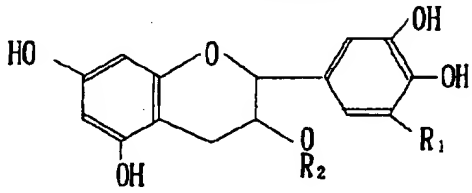
【従来の技術および発明が解決しようとする課題】ヒアルロニダーゼは動物の睾丸や蛇毒、細菌等に存在する酵素であり、動物の結合組織に広く分布しているヒアルロン酸の加水分解酵素である。ヒアルロニダーゼは起炎症＊剤としての作用を持つことが知られている。一方、抗炎症剤や抗アレルギー剤により活性が阻害されることから、ヒアルロニダーゼ活性を阻害することにより、炎症やアレルギーを軽減することが可能であると考えられている。さらには、クリーム，乳液，口紅，ヘアー製品などの化粧品に混合されたヒアルロン酸の分解を防ぐことも期待される。そこで、本発明者らはヒアルロニダーゼの活性化を阻害し、かつ人体に対して有害な副作用を有さない酵素阻害剤の開発を試みた。

【０００３】

【課題を解決するための手段】本発明者らは化学合成品でなく、天然物の中から目的とする薬効を有する物質を検索すべく鋭意研究を重ねた結果、茶および茶ポリフェノール類に該物質が含まれていることを見出し、本発明に到達した。すなわち、本発明は茶ポリフェノール類を有効成分として含むヒアルロニダーゼ活性阻害剤を提供するものである。本発明のヒアルロニダーゼ活性阻害剤の主成分である茶ポリフェノール類は、下記の一般式Ｉで表される茶カテキン類と一般式IIで表される茶テアフラビン類である。

【０００４】

【化１】

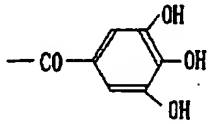


…Ｉ

【０００５】（式中、R_1 はＨまたはＯＨを示し、R_2 はＨまたは

【０００６】

【化２】



【０００７】を示す。）

【０００８】上記の一般式Ｉで表される茶カテキン類の具体例としては以下のものを挙げることができる。

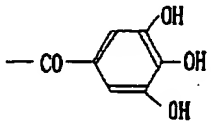
（－）エピカテキン（一般式Ｉ中、R_1 ＝Ｈ，R_2 ＝Ｈのもの）

（－）エピガロカテキン（一般式Ｉ中、R_1 ＝ＯＨ，R_2 ＝Ｈのもの）

（－）エピカテキンガレート（一般式Ｉ中、R_1 ＝Ｈ，R_2 ＝

【０００９】

【化３】

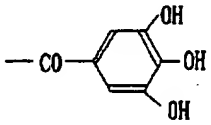


【００１０】のもの）

（－）エピガロカテキンガレート（一般式Ｉ中、R_1 ＝ＯＨ，R_2 ＝

【００１１】

【化４】

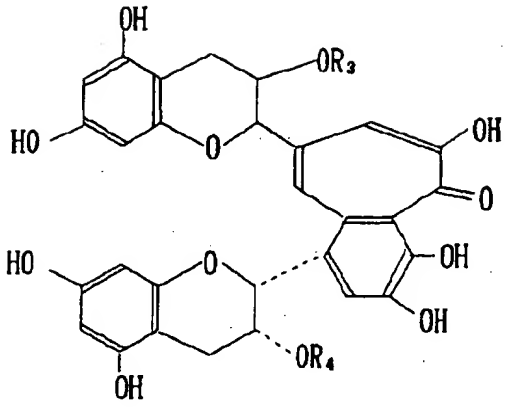


【００１２】のもの）

一般式II

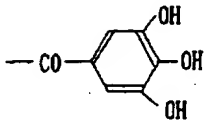
【００１３】

【化５】

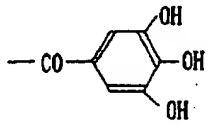


…II

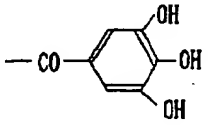
【００１４】（式中、R₃及びR₄はHまたは
【００１５】
【化６】



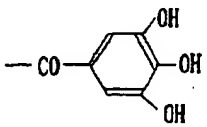
【００１６】を示し、R₃及びR₄は同じであっても異なっていてもよい。）
【００１７】次に、上記の一般式IIで表される茶テアフラビン類を具体的に示すと、以下のものがある。
遊離型テアフラビン（一般式II中、R₃＝H，R₄＝Hのもの）
テアフラビンモノガレートＡ（一般式II中、R₃＝
【００１８】
【化７】



【００１９】，R₄＝Hのもの）
テアフラビンモノガレートＢ（一般式II中、R₃＝H，R₄＝
【００２０】
【化８】



【００２１】のもの）
テアフラビンジガレート（一般式II中、R₃，R₄＝
【００２２】
【化９】



【００２３】のもの）
上記茶ポリフェノール類は茶葉を原料として製造することができ、その製法は特開昭５９－２１９３８４号公報，同６０－１３７８０号公報，同６１－１３０２８５号公報などに記載されている。
【００２４】本発明のヒアルロニダーゼ活性阻害剤は、単独で使用する他、適当な賦形剤、例えばゼラチン，アルギン酸ナトリウムなどと混合したり、水，アルコール類などの溶媒、カルボキシメチルセルロースなどの希釈剤等と組合わせて用いられる。本発明のヒアルロニダーゼ活性阻害剤の使用量については、薬剤として人体に投与する場合、通常は１日量が0.５～１０ｇ、好ましくは１～３ｇ程度となるように経口的に服用すればよい。剤形は任意で散剤，錠剤，カプセル剤などとして用いる。また、化粧品などに配合する場合は、最終濃度が１～１００ppm となるように添加すればよい。
【００２５】
【実施例】次に、本発明を実施例により詳しく説明する。なお、阻害能の測定は常法に基づき次の方法で行った。ヒアルロニダーゼ活性阻害剤溶液（１mM）５０μl に酵素（from Bovine testis, SIGMA 社製）溶液100 μl (2,000 unit/ml緩衝液）を加え、３７℃で２０分間放置する。次に、酵素活性化剤（商品名：compound 48/80, SIGMA 社製）溶液（0.1mg/ml緩衝液）１００μl を加え、３７℃で２０分間放置した後、基質であるヒアルロン酸（from rooster comb, WAKO 社製）溶液（0.8 mg/ml 緩衝液）２５０μl を入れ３７℃で４０分間放置した。0.4N NaOH １００μl を加えて反応を停止させた後、Morgan-Elson法の変法（Davidson,E. A.,Aronson, N. N.: J.Biol. Chem. 242, 437(1967)）により生成物の定量を行い、ヒアルロニダーゼ活性阻害能を求めた。阻害活性は次の式から求められる阻害率で表した。また、緩衝液には0.1 M 酢酸緩衝液（pH 4.0）を用いた。
【００２６】
【数１】阻害率(%)=[(A-B)-(C-D)]/(A-B) ×100
【００２７】A:対照溶液の５８５nmにおける吸光度
B:対照溶液blank の５８５nmにおける吸光度
C:阻害剤溶液の５８５nmにおける吸光度
D:阻害剤溶液blank の５８５nmにおける吸光度
【００２８】実施例１
ヒアルロニダーゼ活性阻害剤として第１表に示した各種

茶ポリフェノール類を使用し、上記した方法で各物質のヒアルロニダーゼ活性阻害能を求めた。結果を第１表に示す。

＊【００２９】
【表１】
＊

第　１　表

阻害剤	阻害率(%)
(+) カテキン	26.8
エピカテキン	22.1
エピガロカテキン	17.6
エピカテキンガレート	55.5
エピガロカテキンガレート	40.3
遊離型テアフラビン	12.2
テアフラビンモノガレートＡ	85.0
テアフラビンモノガレートＢ	86.2
テアフラビンジガレート	99.1

【００３０】表から明らかなように、カテキン類とテアフラビン類の中ではエピカテキンガレート，エピガロカテキンガレート及びテアフラビンモノガレートＡ，テアフラビンモノガレートＢ，テアフラビンジガレートが強いヒアルロニダーゼ活性阻害能を持つことが確認された。

【００３１】
【発明の効果】本発明のヒアルロニダーゼ活性阻害剤　※は、日常相当量飲用されている茶から抽出される天然物を主成分とするため、薬剤としても人体に対する副作用の心配がない。しかも、本発明のヒアルロニダーゼ活性阻害剤は低濃度の添加でヒアルロニダーゼの活性化を著しく阻害する。したがって、本発明のヒアルロニダーゼ活性阻害剤は医薬として各種炎症やアレルギーを軽減するために用いる他、化粧品等に添加してヒアルロン酸の分解を防ぐことができる。

フロントページの続き

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Ａ６１Ｋ　7/48		9051－4C		
Ｃ０７Ｄ 311/62		7252－4C		